

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Validation of RyhB repression of *ynfF*. (A) Schematic of *lacZ* translational fusion for *ynfF*, a gene predicted by Ribo-seq to be repressed by RyhB. A constitutive promoter from pAMD001 was fused to the region around the start of the *ynfF* gene and this was fused to *lacZ* in a single-copy plasmid. (B) β -galactosidase assays of the *ynfF lacZ* fusion. Data are shown for RyhB⁻ (MG1655 $\Delta lacZ \Delta rhyB$; dark gray bars) and RyhB⁺ (MG1655 $\Delta lacZ$; light gray bars) strains. β -galactosidase activity was normalized as described in the Materials and Methods.

Figure S2. Validation of genes predicted by Ribo-seq to be weakly activated by RyhB. (A) Schematic of *lacZ* translational fusions. Regions upstream of candidate genes, including the first 24 bp of the gene, were fused translationally to *lacZ* in a single-copy plasmid. (B) β -galactosidase assays of *lacZ* fusions for genes predicted from Ribo-seq data to be weakly repressed by RyhB. Data are shown for RyhB⁻ (MG1655 $\Delta lacZ \Delta rhyB$; dark gray bars) and RyhB⁺ (MG1655 $\Delta lacZ$; light gray bars) strains. β -galactosidase activity was normalized as described in the Materials and Methods.

Figure S3. RyhB directly activates translation of *cirA* by base-pairing with the 5' UTR. (A) Prediction of base-pairing interactions between RyhB and the *cirA* 5' UTR. The arrows indicate the changes in the mutant RNAs. (B) β -galactosidase assays of wild-type and mutant *cirA* translational fusions to *lacZ* in cells expressing wild-type or mutant RyhB, as indicated. β -galactosidase activity was calculated as described previously (60).